FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
1390-0129P	NEW 10/089541

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FI00/00914	20 October 2000	21 October 1999

TITLE OF INVENTION

A TEST STRIP PROVIDED DEVICE WITH A LID-PROVIDED PRETREATMENT PORTION

APPLICANT(S) FOR DO/EO/US

SVENS, Helena Eivor

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail Label No. EL533910833US
23. ☒ Other items or information:

Ten (10) Sheets of Formal Drawings
Application Data Sheet
Letter Submitting Article 34 Amended Claims

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
NEW 10/089541	PCT/FI00/00914	1390-0129P

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$1,040.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	40 - 20 =	20	x $18.00	**$360.00**
Independent claims	1 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable).**			☒	**$280.00**

TOTAL OF ABOVE CALCULATIONS =	**$1,810.00**
☒ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.	**$905.00**
SUBTOTAL =	**$905.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	**$0.00**
TOTAL NATIONAL FEE =	**$905.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	**$0.00**
TOTAL FEES ENCLOSED =	**$905.00**
Amount to be: refunded	$
charged	$

a. ☒ A check in the amount of **$905.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-2448** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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REGISTRATION NUMBER

29 March 2002
DATE

A TEST STRIP PROVIDED DEVICE WITH A LID-PROVIDED PRETREATMENT PORTION

The Technical Field of the Invention

The present invention is related to a test device with a lid-provided pretreatment portion mounted on the same backing support as a test strip and having means for controlled regulation of sample and diluent flow. A method for directly carrying out assays from samples generally requiring more or less time consuming pretreatment procedures with said device is also disclosed.

The Background of the Invention

Some biological samples, especially such samples which are taken for making diagnoses from whole blood, serum, urine, feces, saliva, sputum, synovial fluid, etc. require pretreatment procedures including removal of particles, agglutination, chemical treatments, release of specific components, immunocapture, etc.

Usually, before carrying out the test, a whole blood sample is coagulated and centrifuged in order to remove blood cells and other interfering or disturbing factors. Many novel and rapid bed-side tests have been developed and they would be perfect for making rapid bed-side tests in emergency situations in ambulances and in hospitals during chirurgical operations. However, the centrifugation is a retarding factor which hampers the use of said tests in really critical situations. Many systems for removing blood corpuscles on a test strip or test device have also been disclosed previously (EP 806 666, EP 323 605, EP 582 231 and WO 98/22824).

Some problems are related to said known systems. Said problems

are for example backflow of diluting buffers and overflow, i.e. redundant fluid may pass the edges of the filtering means, leaving the reagents in the reagent layer and causing disturbances in the detection zone. Another problem connected with said known methods and devices is that only one filtering pad or layer is often not sufficient to retain all blood cells and all interfering factors are not retained either.

Thus, the objective of the present invention is to provide a test device with a test-strip and improved lid-provided pretreatment portion having means for regulating the sample and diluent flow. Said device is useful in emergency situations, especially for use in ambulances wherein coagulation and centrifugation is not possible to carry out or in which said steps are too time consuming. On the test device of the present invention even complicated immunological analysis can be performed rapidly with great accuracy without pretreatment of the sample. Furthermore, the test device can be modified to meet the requirements in a multitude of different test methods.

The Summary of the Invention

The characteristics of the analytical test device with a closed pretreatment system assembled with a test strip are defined in the claims. More specifically, the invention relates to a test device for obtaining a controlled regulation of sample and diluent flow. Said test device has a pretreating portion for treating the sample and removing interfering substances and particles before performing the assays on a test-strip placed on the backing support (1). The pretreatment portion is provided with a lid (2) having an aperture (3). Said lid covers and protects the pretreatment system having one or more pretreatment layers (4) horizontally stapled upon each other and assembled in capillary flow contact with the test strip (5), which is placed on the lid-provided backing support (1). The backing support (1) and the lid (2) are

provided with means for keeping the layers (4) in the pre-treatment system in their correct positions and to connect them with the test strip (5). Said means for securing and fixing the position of the layers are responsible for a controlled regulation of the flow of the sample solution. Said means also form an excess liquid collecting compartment (6), which enables rapid addition of buffer or other liquids in order to allow more efficient dilution of sample and reagents impregnated into the layers. The compartment (6) minimizes the negative effects of overflow and backflow and enables an even and controlled passage through each layer in a predetermined order and subsequently into the test strip (5) where the result is recordable as one or more visable or readable zones.

Moreover, the invention relates to a method for carrying out a rapid bed-side or field test without pretreating the sample. The sample is added into the aperture (3) in the lid (2) on the backing support (1) of the test device and a diluent or aqueous driving solution is added. The diluent may contain agents, which are essential for properly carrying out the test. The diluent which may be a buffer or pure water is capable of driving the sample solution and diluents through the layers in the pretreatment system in a controlled manner. Particles are captured and interfering substances are removed or allowed to react by using physical and/or chemical means in the layers of the pretreatment system. Superfluous or excess fluid is collected in the compartment formed by the bar (9.1) in the lid and the flanking support (8.1) and the bar (9.4) in the lid-portion of the backing support.

A Brief Description of the Drawings

Fig. 1 is a side view of a lid-provided backing support (1) and the lid (2) with a hinge (A). The lid is snapped in place with the means for attaching the lid (C) to protect the layers in the pretreatment system.

Fig. 2 is a schematic view from above of the test device with a closed lid (2) snapped on the backing support (1) and the test strip (5) fixed in its correct position. The lid (2) is provided with a shaped or non-shaped aperture (3) into which the sample solution and possible diluents or driving solutions can be added.

Fig. 3 is a sectional side-view of the lid-provided backing support (1) with the lid (2) open. The aperture (3) for adding sample solution is seen as an intersection. Two pretreatment layers (4.1) and (4.2) as well as the test strip (5) are schematically shown.

Fig. 4 shows a view from above of the lid-provided backing support (1) with the lid (2) open and the aperture (3) shown as a round dot or spheric area and with the pretreatment layers (4.1) and (4.2) as well as the test strip (5) placed in their correct positions.

Fig. 5 is a sectional side view of the lid-provided backing support (1) with the lid (2) snapped on the backing support (1) and covering and protecting the pretreatment layers (4.1) and (4.2). The pretreatment layers are in capillary flow contact with the test strip through the conjugate pad (B). All layers are placed in their correct positions.

Fig. 6 is a sectional view seen from above with a transparent closed lid (2) and the aperture (3) snapped on the backing support (1). Due to the transparency the lid-covered pre-treatment layers (4), which normally are not seen, can be seen in their correct positions and in capillary flow contact with the test strip (5). The transparency is not a prerequisite in the present invention. Transparency was used during developmental work for experimental purpose to enable studies of what is going on in the different layers.

Fig. 7 is a sectional side view of the lid-provided backing

support (1) with the lid (2) open and without pretreatment layers and without the test strip. In the Figure, the means for securing and fixing the layers in their correct positions are shown. These means include taps (7), flanking supports (8) and bars (9).

Fig. 8 is a schematic picture viewed from above of the lid-provided backing support (1) with the lid (2) open without pretreatment layers and without the test strip. The taps (7), the side wall protrusions or flanking supports (8), the bars (9) as well as the area forming the compartment (6) acting as a reservoir for the excess fluid.

Fig. 9 is a sectional side view of the lid-provided backing support (1) with the lid (2) snapped in place without pretreatment layers and test strip. In this Figure the space provided for the layers is clearly indicated.

Fig. 10 is a sectional view seen from above with a transparent closed lid (2) snapped on the backing support (1) and without the pretreatment layers and without the test strip. The aperture (3) is shown in this Figure as well as the taps (7) and the side wall protrusions forming the flanking supports (8). The bar (9.4) which forms the reservoir of the compartment is clearly indicated.

The Detailed Description of the Invention

Definitions

In the description which follows, most terms are used in the same way they are generally used in relation to methods and devices used in diagnostics, immunochemistry and biochemistry and enzymology. However, some terms are used in a somewhat different or more extensive way. In order to provide a clearer and more consistent understanding of the specification and claims including the scope to be given such terms, the

following definitions are given.

In the present invention the term "means for securing and fixing the position of the layers" are responsible for a controlled regulation of the flow of the sample solution and appropriate diluents. They also form a compartment for collecting excess fluid or liquid. This compartment allows efficient dilution of reagents. It minimizes the effects of overflow. Simultaneously, negative backwash effects are avoided. The compartment enables an even and controlled passage through each layer in a predetermined order and subsequently into the test strip. The "means for securing and fixing the position of the layers" are provided by taps, flanking supports and bars, which are longer or shorter protrusions present in the plastic lid and the lid-portion of the backing support of the device. The means have somewhat different functions.

The term "backwash effect" and/or "backflow effect" means the phenomenon when the sample solution and the liquid, acting as a diluent, drives the sample solution in the wrong direction, i.e. in a direction opposite to the intended one. In the worst case, the sample flows over the reaction area and the reagents are washed back. In the present invention, uncontrolled backwash is not possible. The negative backwash effect is abolished, because the excess fluid is collected behind the pretreatment layers in a compartment, which is later emptied, when the excess fluid from the compartment migrates through the filter layers into the conjugate pad and the reagent area of the test strip by capillary forces of the absorbing pad in the opposite end of the test strip.

The term "tap" means smaller protrusions, such as pegs or tags on the bottom of the plastic backing of the device. Said taps support the filtering means and prevent them from lying directly on the backing support. Alternatively, a grid can be used. Thus, the sample solution and the diluent cannot flow

along the backing support. In other words, the sample solution is prevented from passing into the test strip without first passing the filters.

The term "flanking support" means protrusion in the backing support which keep the pretreatment layers in fixed positions. One flanking support in the rear of lid-portion of the backing support assists in forming the excess fluid compartment. The flanking supports in the side walls also prevent excess fluid from migrating into the test strip without first passing the pretreatment layers.

The term "bars" mean ridges and ribs which can be "toothed" in order to provide a better grip. Such a "toothed bar" is present in the lid and keeps the test strip in a firm grip. The bars may also form the wall of the compartment for excess fluid and prevents the excess fluid from flowing under the filter layers. The bars together with the flanking supports also fix the pretreatment layers in their correct positions.

The term "test strip" and/or "test stick" means a laminated strip or stick comprising for example a nitrocellulose or nylon membrane mounted on a backing. The test strips or sticks are provided with reagents preferably immunoreagents such as mono- or polyclonal antibodies and further provided with recordable or visible markers or labels. The test sticks can alternative be enzymatic, chemical or biochemical.

The term "aperture" means a shaped or non-shaped hole for adding the sample and/or diluent.

The General Description of the Invention

The objective of the present invention is made feasible by providing an analytical test device, comprising a system for pretreating samples before carrying out an immunochromatographic test.

In one preferred embodiment of the present invention the test strip is an immunochromatographic test producible as follows:

A nitrocellulose or nylon membrane is mounted on a plastic backing or between plastic strips and is provided with a conjugate pad in close contact with one end of a nitrocellulose or nylon membrane and with an absorbent pad attached to the other end of the membrane. A narrow zone on the nitrocellulose or nylon membrane is coated with a monoclonal antibody against a specific component. Coloured or fluorescent latex particles, as well as colloidal particles, gold sols, magnetic particles, etc. may be coated with another antibody preferably a monoclonal antibody against the same component. The coated particles are dried on a zone preferably close to the pretreatment portion of the strip or in a layer placed in the pretreatment portion. The diameter of the particles is so small that they can flow freely through the pores and strip materials. The layers and the test strips are placed on a plastic backing so that they are in a capillary flow contact of the sample liquid to test strip through the appropriate filter layers.

The test strip is, however, not restricted to the test strip embodiment described above. A multitude of different test devices and determinations which require pretreatment of the sample can be used in the present test device including immunoassays as well as enzymatic, chemical or biochemical test strips.

The test device of the present invention is provided with a lid-provided pretreatment area, comprising one or more layers, preferably of a hydrophilic, bibulous material, stapled upon each other. They provide means for physical or chemical treatment of the sample. Said pretreatment area is provided with a lid or cover of plastic material with good wetting properties and means for keeping the layers fixed in a predetermined

position with each others and with the test strip.

The cover or lid is optionally loose or attached to the backing support by fastening means such as hinges or pivots. The fastening means can be placed on any side of the backing support, but the most preferable place is in the near, i.e. at the outer or upper end of the backing support. If the hinges are placed on either side, the flow of the sample solution may not be even, i.e. it can be different on different sides of the layers. When the pretreatment layers and the test strip are assembled the lid is snapped over the pretreatment portion.

The layers in the pretreatment system comprise one or more different layers, which allow physical as well as chemical pretreatment of the sample. Said physical treatment includes separation or removal of certain components or particles or means for regulating the mobility of the components in the sample solution. In order to enable the physical treatment, filters or membranes with different pore sizes or with shaped pores are used. Alternatively, filters having different so called V-pores, i.e. having pores with different diameters on each side of the filter or filters having different pore sizes on each side are used for separating particles of different sizes in the sample solution.

The layers in the pretreatment portion can in addition to providing physical treatment comprise means for chemical treatment of the sample. Said means for chemical treatment are filters or membranes containing certain compounds or compositions acting as agglutinating, coagulating, lytic, buffering and ionic strength regulating agents as well as immunocapturing agents. The layers can also be used as carriers for so called labels or markers, including coloured, phosphorescent or fluorescent latex particles, colloids, gold sols, liposomes, etc. It is also possible to add chemical substance, which are capable of releasing specific components from the

substances which are to be determined.

The test device comprises a supporting back preferably prepared by good quality, wettable, plastic material, such as polypropens. Because of the hinges or pivots, it is essential that the material is substantially non-brittle. Furthermore, the material should not contain any disturbing chemicals. It is for example not recommendable to use mold releasing agents or plasticizers for the preparation of the lid and backing support. Surface treatments are not recommendable.

The backing support is provided with a lid preferably made of the same material as the backing support. The lid covers the pretreatment portion and simultaneously fixes the test strip in capillary flow contact with the pretreatment layers. The sample solution, has to pass all the required layers before entering the test strip by capillary flow. The backing support and the lid of the test device act as a protector for the test strip during storage and transport. Otherwise the test strip itself is not covered.

The inside of the lid, as well as the pretreatment portion of the test device, is provided with means, including taps (7), flanking supports (8) and bars (9), which fix the layers of the pretreatment portion firmly with each others and the test strip. The contact between the test strip and the pretreated sample solution is made feasible only through the aperture in the lid. The sample solution flows by capillary forces through the pretreatment layers into the test strip.

The lid is constructed to enable a firm capillary flow contact between the sample solution and the test strip through the pretreatment layers, e.g. the filters in the desired and predetermined order. The pretreated sample solution migrates only through the pretreatment layers into the test strip. Redundant or excess fluid is temporally collected in a compartment (6) formed by the bar (9.4) in the lid portion of the

backing support.

The test strip can be placed into the test device during the manufacture and sold as a ready to use disposable kit. Alternatively, the test strip, test device and layers for the pretreatment portion can be sold separately and assembled in desired manner before use.

The sample can be whole blood, serum, urine, feces, saliva, sputum, synovial fluid, amniotic fluid, but also environmental samples of different forms. Generally, it is essential that particular and/or solid material can be removed from the sample. This can be achieved with filtering means such as a pad with suitable pore sizes. Sometimes, the sample has to be chemically treated in order to separate interfering or disturbing components. Sometimes, some specific or active components in the substances to be determined from the sample have to be released before they can be determined. Such components are for example epitopes or active sites in certain proteins or haptens. The release can be carried out e.g. by extraction using different reagents, such as detergents, reducing agents, acids, etc. The agents remove for example sulphur-bridges, lipid, etc.

The test device is preferably used with samples requiring different kinds of pretreatments. In close contact with the test strip, the lid-provided portion of the test device may contain one layer of material, or several layers of the same material or of different types of materials. These materials can e.g. have different pore sizes, and may be used as prefilters. They can be impregnated with different kinds of reagents and they may act as reagent layers or as immunocapture layers. These layers may be used separately or in any combination with each other.

When the sample specimen is whole blood, separation of blood cells is usually required. This can be achieved by preferable

using two layers of material in close contact with the lateral flow test strip. The upper layer is preferably acting as a sample pad. The sample pad together with the underlaying filter separates blood cells from whole blood allowing plasma or sera to migrate forward to the test strip.

When the sample specimen is for example serum, which may contain e.g. rheumatoid factors, heterophilic anti-mouse antibodies (HAMAs), heterophilic anti-animal antibodies (HAAAs) or the like, the lid-provided portion may contain layers impregnated with reagents for eliminating these interfering substances. Alternatively, the serum specimen can be applied to a layer acting as a sample pad and eluted with a buffer containing such reagents. In fact, it may be essential for the performance of the test device that a driving solution, either water or preferably a buffer is added. The driving solution dissolves and mixes the sample and reagents and drives them through the pretreatment layer(s) into the test strip and the zone where the result can be read. However, the buffer is known to cause problems such as backflow. The present invention solves said backflow problem by collecting any liquid flowing backwards into the compartment behind the filter(s), but thereafter the compartment is efficiently emptied by capillary forces and all sample and reagents are transferred to the test strip.

When the sample specimen is urine or a suspension of feces, the lid-provided portion may contain one or more layers of filters with the same or with different pore sizes. A prefilter with a coarse pore structure may lay on the top of one pretreatment layer with a fine pore structure. Large and small particles can subsequently be filtered away before the sample liquid reaches the test strip.

When the sample specimen is a urine sample, having for example a very low pH value, caused e.g. by a preservative, or a very low ionic strength, it may preferably be pretreated in one or

several buffered layers before the sample liquid reaches the test strip.

When the sample specimen is saliva, sputum, synovial fluid or amniotic fluid it may be preferable to use mucous dissolving agents impregnated into the layers of the pretreatment portion.

The sample is added to the test device through the aperture in the lid of the pretreatment portion. The volume of the sample can be such, that no additional reagent solution is needed. In cases where the sample volume is very small, a diluent solution, preferably an aqueous buffer is necessary in order to get a flow of liquid from the pretreatment portion to the end of the test strip in the device.

The addition of sample liquid to the opening in the lid is preferable added drop-wise. The first drop of liquid spreads through the top layer of the filters, i.e. the hydrophilic, bibulous sample pad. The further drops flow through the sample pad into the underlaying filter layer and spread horizontally into the back compartment (6) in the lid-portion of the backing support. The filter materials are all in close contact with each other, and with the bibulous filter part or conjugate pad (B) of the test strip (5). The pretreatment layers or filters are laying on taps (7) in the plastic device, and they are in turn held in place by flanking supports (8) in such a way, that the liquid is forced to flow through the filter and/or reagent layers in a predetermined order and not along the inner surface of the plastic device.

The sample liquid with or without the diluent or driving solution is spread along the underlaying filter(s) (4), and wets the end of the test strip (5), i.e. the conjugate pad (B). The excess fluid collecting compartment (6) of the lid-portion in the rear end of the backing support is emptied as the liquid flows forward along the conjugate pad (B) and

further into the membrane part of the test strip driven by capillary forces which are provided by the absorbant pad in the opposite end of the test strip (5).

Microspheres, e.g. latex particles covered with an antibody, dried upon the conjugate pad redissolve, and migrate forward with the liquid front into the reaction area on the membrane of the test strip. The absorbent pad in contact with the membrane of the test strip absorbs excess liquid and ensures that the compartment (6) will be emptied.

The sample is preferably pipetted into the hole or aperture on the cover or lid of the test device and optionally a suitable buffer solution is added for driving the sample through the layers.

The solution is forced through a first filter layer which removes greater components or particles into the following layer. Behind the layers is a compartment (6) into which excess liquid can be collected so that it is not forced beside and over the pretreatment layers (4) into contact with the nitrocellulose or nylon membrane of the test strip (5). Furthermore, the sides of the backing support (1) and the lid (2) is provided means (7, 8 and 9) for attaching the different layers in fixed positions. The means provided are for example in form of a grid lattice or more preferably in the form of taps (7). The grid or taps (7) are supporting the pretreatment layers so that they do not touch the backing support (1). The flanking supports (8) prevent the sample solution from passing along the sides of the filters. Furthermore, the lid (2) and support (1) is provided with bars (9). The lid (2) is provided with a toothed bar (9.3) which keeps the test strip in place. The lid-portion of the backing support is also provided with a bar (9.4), which assisted with the flanking support (8.1) forms the compartment (6) for redundant or excess fluid.

Thereafter, the test is allowed to develop without any pos-

sibly disturbing movements until the result is visible or readable. The result is recorded directly. It is preferable that the amount of sample and diluent is such that solution is absorbed and not left in the excess liquid collecting compartment (6).

The test device of the present invention and the use thereof for performing analyses with a test strip or test stick is described in more detail by referring to the attached Figures 1-10, wherein the reference numbers and/or letters used refer to the corresponding features independent of the design of the test device.

In this connection it should be understood that the following description and Figures are intended to be examples, which should in no way restrict the invention to the specific features shown in the Figures. On the contrary, the scope of protection is intended to cover all modifications, equivalents or alternatives, which contain the characteristics of the device as defined in the claims.

Fig. 1 is a side view of a lid-provided backing support (1) with an edge and the lid (2) snapped in place with the means for closing (C) the lid by snapping to protect the layers in the pretreatment system. The backing support and the lid portion are connected with suitable fastening means, such as hinges (A) or pivots placed in the rear of the test device in the most preferred embodiment of the present invention.

Fig. 2 is a schematic picture of the closed lid (2) snapped on the backing support (1) and with the pretreatment layers (not shown) hidden under the lid and the test strip (5) fixed in their correct position. The lid (2) is provided with a preferably shaped aperture (3) into which the sample solution and a possible diluent or driving solution can be added and also with means for closing (C) the lid.

Fig. 3 is a sectional side-view of the lid-provided backing support (1) with the lid (2) open. The aperture (3) for adding the sample solution is shown as an intersection and two pre-treatment layers (4.1) and (4.2), which include for example a first filter pad (4.1) and a second filter pad (4.2) and the bibulous area of the test strip (5) are schematically shown as well as the fastening means or hinge (A) in the rear of the test device. The conjugated area in which the filter (4.2) is in capillary flow contact with test strip (5) is indicated with the letter (B) and the means for closing (C) the lid by snapping over the pretreatment portion is indicated with (C). Also shown are taps (7) and bars (9.1), (9.2) and (9.3) which support the layers in the pretreatment portion and a flanking support (8.1) and a bar (9.4) which form a compartment (6) or reservoir basin for excess or redundant fluid.

Fig. 4 shows a view from above of the lid-provided backing support (1) with the lid (2) open, the aperture (3) for adding sample and with the pretreatment layers (4.1) and (4.2) and the test strip (5) placed in the correct positions. The flanking support (8.1) and bar (9.1) forming the reservoir compartment (6) as well as the side wall protrusions or flanking supports (8.2) preventing excess fluid from passing around the filters and the bars (9.1), (9.2) and (9.3) which fix the layers are also indicated. The fastening means or hinge (A) and filter layer-test strip-connecting area, the conjugate pad (B) as well as the snapping or closing means (C) are schematically shown. An enlarged view of one preferred embodiment of the toothed bar (9.3), which prevents the test strip from moving, is shown in detail seen from the rear end of the lid and the backing support.

Fig. 5 is a sectional side view of the lid-provided backing support (1) with the lid (2) with the aperture (3) snapped on the backing support and covering and protecting the pretreatment layers (4.1) and (4.2) and connected with the test strip (5), all placed in their correct positions. The fastening

means or hinge (A) and the connection area, conjugate pad (B) between the filter (4.2) and test strip (5) as well as the snapping region (C) are also schematically shown. Also shown are the taps (7) which support the layers as well as the flanking support (8.1) and the bar (9.4) which form the compartment (6). The compartment area collect excess fluid and prevents it from passing around the filters.

Fig. 6 depicts a view seen from above with a closed transparent lid (2) and the aperture (3) on the backing support (1). Also seen are the layers of which (4.1) is a prefiltering pad and the filter (4.2) is connected with the test strip (5). Side wall protrusions and/or flanking supports (8.2) are also shown on each side of the filter layers as well as a flanking support in the rear end (8.1). The area marked (B) indicates the filter-test strip connecting area, the conjugate pad and (C) the snapping area.

Fig. 7 is a cross-sectional side view seen from one side in longitudinal direction of the lid-provided backing support with the lid portion (2) open and without pretreatment layers and test strip. The lid portion (2) is attached to the backing support (1) with hinges (A), which preferably are placed in the rear of the lid portion of the backing support (1) and not on either side of the lid portion in order to avoid uneven mobility or flow of the sample fluid. The backing support comprises two portions. The lid portion being the sample pretreatment portion is covered by the lid (2) and the assay portion carries the test strip (not shown). The lid (2) is provided with a shaped aperture (3) and the backing support with a side wall in the backing support (1.1) the inside of which is shown in this Figure. The lid (2) is snapped to the backing support (1) and the bars (9.1), (9.2) and (9.3) which can be of different heights and breadths can be used to fix the filtering layers. They act as fastening and supporting means for the layers and the test strip (not shown). They can also be placed so that they form a separate compartment (6)

for collecting excess or redundant sample fluid and enable an even flow into the test strip or test membrane.

The backing support (1) of the lid portion is also provided with flanking supports (8.2) and/or taps (7) of different heights which are adjusted so that they support differently shaped and sized filter layers. The filter layers are of different thickness and different sizes (dimensions). The flanking support (8.1) and the bar (9.4) forms the compartment (6) which acts as a reservoir basin for redundant or excess sample fluid.

Fig. 8 is a schematic picture viewed from above of the lid-provided backing support (1) with the lid (2) open without pretreatment layers and test strip. The lid (2) and the supporting back (1) are connected by the fastening means or hinge (A). In the lid (2) the aperture (3) is a shaped hole into which the sample is added or pipetted. The lid (2) is further provided with bars (9.1), (9.2) and (9.3) of different height which keep the different filter layers in desired places. The bar (9.1) in the lid and the bar (9.4) and the flanking support (8.1) in the lid portion of the backing support forms a compartment (6) acting as a reservoir for redundant or excess sample solution. The flanking supports (8.1) and (8.2) and the taps (7) are separating the reservoir of sample solution from the test strip and forces the fluid to pass the appropriate filter layers (not shown).

Fig. 9 is a sectional side view of the lid-provided backing support (1) with the lid (2) closed without pretreatment layers and test strip. The aperture (3) in the lid (2) is shaped to divert the sample and eluting buffer into the filter layers (not shown). Bars (9.1), (9.2), (9.3) and (9.4), flanking supports (8.1) and (8.2) and taps (7) which fix the position of the filters are shown. The side wall (1.1) of the backing support (1) is shown from its inside. The lid (2) and the supporting back (1) are connected by the fastening means

or hinge (A).

Fig. 10 depicts the test device seen from above and with a transparent closed lid (2) snapped by means for closing the lid and keeping it in place i.e. snapping means (C) on the backing support (1) and without the pretreatment layers and the test strip. The hinges (A) are placed in the rear of the test device in the preferred embodiment of the present invention. The aperture (3) as well as the flanking supports (8.2) and the taps (7) can be placed for example as indicated. The flanking support (8.1) and the bar (9.4) forms a compartment (6) for excess or redundant sample solution.

EXAMPLE 1

A rapid test for screening the risk of development of iron deficiency anemia (IDA) during pregnancy from whole blood

Serum ferritin concentration indicates the level of iron stores of the body. Ferritin is an early marker of iron deficiency anemia (IDA) because its concentration decreases before anemia has developed. Prelatent and latent anemia is detectable before a decrease in hemoglobin concentration can be observed.

During pregnancy, serum ferritin decreases towards term. Assessment of ferritin during the first trimester of pregnancy can be used to predict the risk of development of IDA later during the pregnancy.

The rapid test described below for determining ferritin can be used to estimate the need for iron therapy during the pregnancy.

The test was performed on whole blood. The cut-off value was about 40 µg/l (calibrated against WHO 3rd International Standard, code 94/572). The positive test result indicated,

that the risk of developing IDA later in pregnancy was small, and no iron therapy should be needed. The negative test result indicated, that the risk of developing IDA was big, and therefore iron therapy should be recommended.

The test was based on lateral flow immunochromatography using monoclonal antibodies against human ferritin. One antibody was bound to colored microspheres, and another antibody was dispensed onto a membrane solid phase.

The test device was composed of a lateral flow test strip and filters for separation of red blood cells from whole blood mounted in the lid-provided pretreatment portion.

The test device was assembled so that at first the test strip was placed into the plastic device, then the filter layers were mounted into their places, and finally the lid was closed. The test device was packed into aluminium foil pouches together with silica gel bags.

Test performance

10 µl of whole blood was pipetted into the aperture of the lid followed by 3 drops of elution buffer. Red blood cells were retained by the filters while serum migrates further and flows along the test strip by capillary force. The test result was read visually or by a reader 5 minutes after addition of elution buffer. One line (control line) in the test window indicated a negative test result. Two lines (test line and control line) in the test window indicated a positive result.

EXAMPLE 2

A rapid test for screening presence of environmental contamination

Samples for environmental fungal analysis, e.g. analysis for *Stachybotrys chartarum*, were collected from suitable sites

identified by the investigator as representing the contaminated area sufficiently. The sample was taken from a site including building material, other substrate, accumulated dust etc. The sample was transferred into a test tube containing buffer solution and shaken carefully. The sample suspension was then transferred into a test device. The test device consisted of an immunochromatographic test stick for recognizing *Stachybotrys chartarum* and a pretreatment device. The lid-provided pretreatment device was assembled by placing two pads of porous material in the plastic compartment. The first pad was impregnated with reagents that were capable of releasing antigenic cell components present in the fungal cell wall. The second pad was made of filtering material capable of removing large particles of fungal structure. If necessary, one more pad can be added, containing immobilized antibodies that capture components that might cause nonspecific reactions with the antibodies used in the immunochromatographic test stick. Sample suspension in buffer was pipetted into the aperture in the lid. Within 5 minutes, the sample liquid migrated through the pads for pretreatment and along the test strip. When fungal antigen were present a visible line was formed in the test strip and the test was interpreted as positive. In other words, the site inspected was contaminated by the indicator fungus, *Stachybotris chartrum*.

Claims:

1. A lid-provided backing support for a test device for performing assays without separate pretreatment of the sample, comprising a pretreating system mounted on the backing support (1) and covered and protected by a lid (2) with an aperture (3) in a lid-portion, said pretreatment system having one or more layers (4) horizontally stapled on each other and assembled in a capillary flow connection with a test strip (5), c h a r a c t e r i z e d in that said lid and the lid-portion of said lid-provided backing support is provided with means (7, 8 and 9) of different sizes and heights for securing and fixing the positions of the layers of the pretreatment system, said means comprising taps (7) supporting the pretreatment layers (4), preventing the layers (4) from lying directly on the lid-provided backing support (1) and forcing the sample solution and diluent to pass through the pretreatment layers (4) in predetermined order before entering into the test strip (5), side wall protrusions providing flanking supports (8) preventing the pretreatment layers from moving backwards or in side direction, and bars (9), which can be of different heights and sizes, which fix the pretreatment layers and act as fastening and supporting means for the pretreatment layers (4.1) and (4.2) and the test strip (5), and at least one bar (9.4), which forms a compartment (6) which allows excess sample solution and diluent to be collected behind the pretreatment layers and negative backwash effects to be avoided, and said compartment (6) to be emptied by controlled and even flow by capillary forces of the sample and diluent through each layer in predetermined order and subsequently into and along the test strip (5).

2. The lid-provided backing support according to claim 1, c h a r a c t e r i z e d in that the flanking support preventing the pretreatment layers from moving backwards (8.1) is placed in the rear end of the lid-portion of the

lid-provided backing support (1) and assists in the formation of the compartment (6) for excess liquid.

3. The lid-provided backing support according to claim 1, characterized in that the flanking supports (8.2) preventing the pretreatment layers from moving in side directions simultaneously force the sample solution and diluent to move through the layers in predetermined order and prevent them from passing outside the layers along the backing support.

4. The lid-provided backing support according to claim 1, characterized in that the means for securing and fixing the pretreatment layers comprises at least one toothed bar (9.3), which secures the connection between the pretreatment layer and the conjugate pad (B) of the test strip.

5. The lid-provided backing support according to claim 1, characterized in that the pretreatment system comprises one or more layers (4) providing physical and/or chemical means for pretreating the sample.

6. The lid-provided backing support according to claim 5, characterized in that the physical means for separating and/or removing components from the sample solution are provided by filter layers with variable thickness and size.

7. The lid-provided backing support according to claim 5, characterized in that the physical means for separating and/or removing components from the sample solution comprise one or more filter layers having shaped pores with different diameters on each side of the filter layer.

8. The lid-provided backing support according to claim 1, characterized in that the chemical means for treating the sample solution comprise buffering, ionic

strength regulating, agglutinating, disrupting, extracting, immunocapturing, immunocatalytic, coagulating and/or lytic agents as well as catalyzators, labels, markers, enzymes, substrates and/or reagents.

9. A method for carrying out a rapid bed-side or field test with the lid-provided backing support according to any of claims 1-8, which lid-provided backing support comprises pretreatment layers and a test strip, c h a r a c t e r i - z e d in that it comprises the steps
(a) adding a liquid sample through the aperture (3) in the lid (2) placed on the pretreatment layers of the lid-provided backing support;
(b) adding a diluent, which is capable of redissolving from the pretreatment layers the reagents impregnated therein; mixing the sample with redissolved reagents and driving the sample and reagent mixture through the pretreatment layers, whereby particles are captured and interfering substances are removed in a controlled manner;
(c) collecting the excess liquid in the compartment (6) to enable a controlled and even flow through the pretreatment layers into the test strip (5); and
(d) recording the visible or readable result in the test strip.

10. The use of the lid-provided backing support for a test device according to any of claims 1 to 8 for assessing ferritin from blood.

11. The use of the lid-provided backing support for a test device according to any of claims 1 to 8 for screening the risk of developing iron deficiency anemia.

12. The use of the lid-provided backing support for a test device according to any of claims 1 to 8 for screening presence of environmental contaminants.

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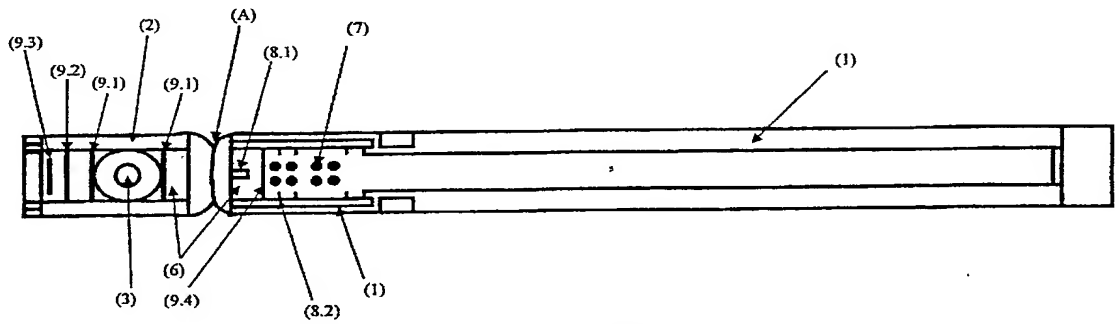
(54) Title: A TEST STRIP PROVIDED DEVICE WITH A LID-PROVIDED PRETREATMENT PORTION



(57) Abstract: The present invention is related to a test device provided with a pretreatment portion covered by a lid (2) with an aperture (3), which is fastened with hinges (A). The pretreatment portion is mounted on the same backing support (1) as a test strip (not shown). The lid (2) and the lid portion of the backing support is provided with means (7, 8 and 9), which support, secure and fix the position of the pretreatment layers, form a compartment (6) for collecting excess sample and regulate the flow of sample solution and diluent. The test device is useful in field tests and bed-side methods, especially in emergency situations when a rapid result is needed.

WO 01/29558 A1

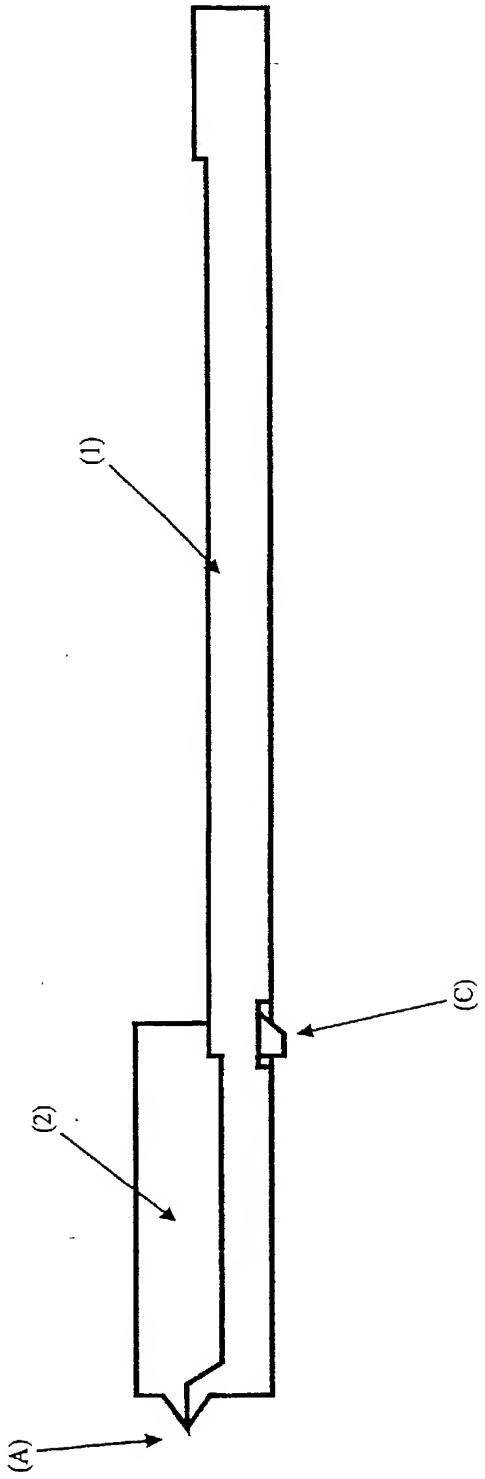


FIG. 1

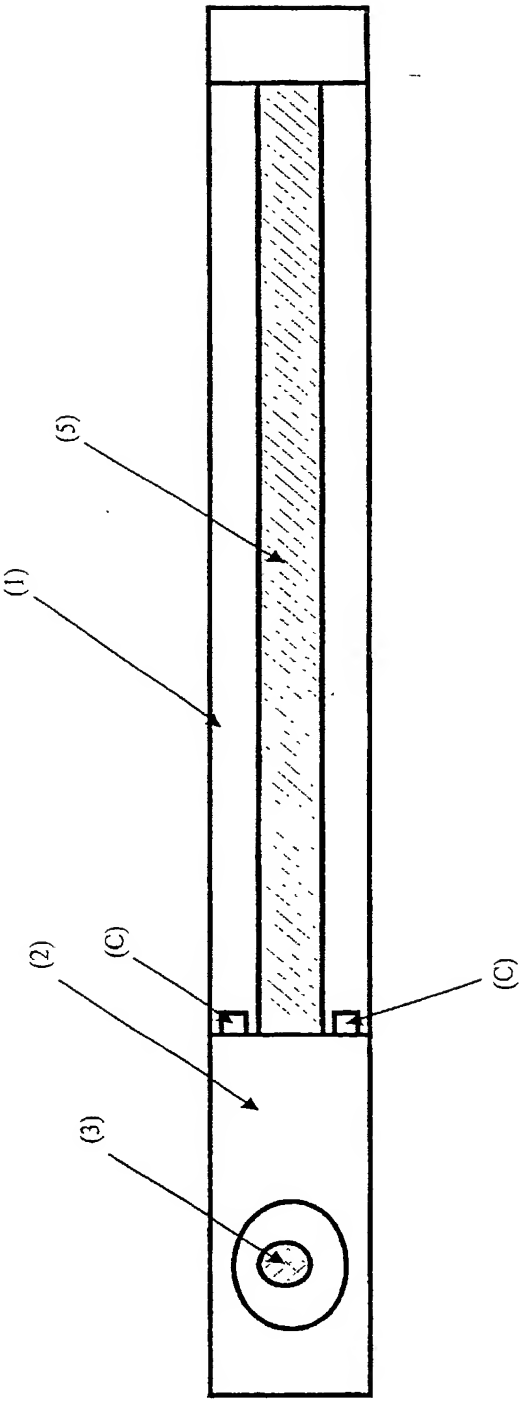


FIG. 2

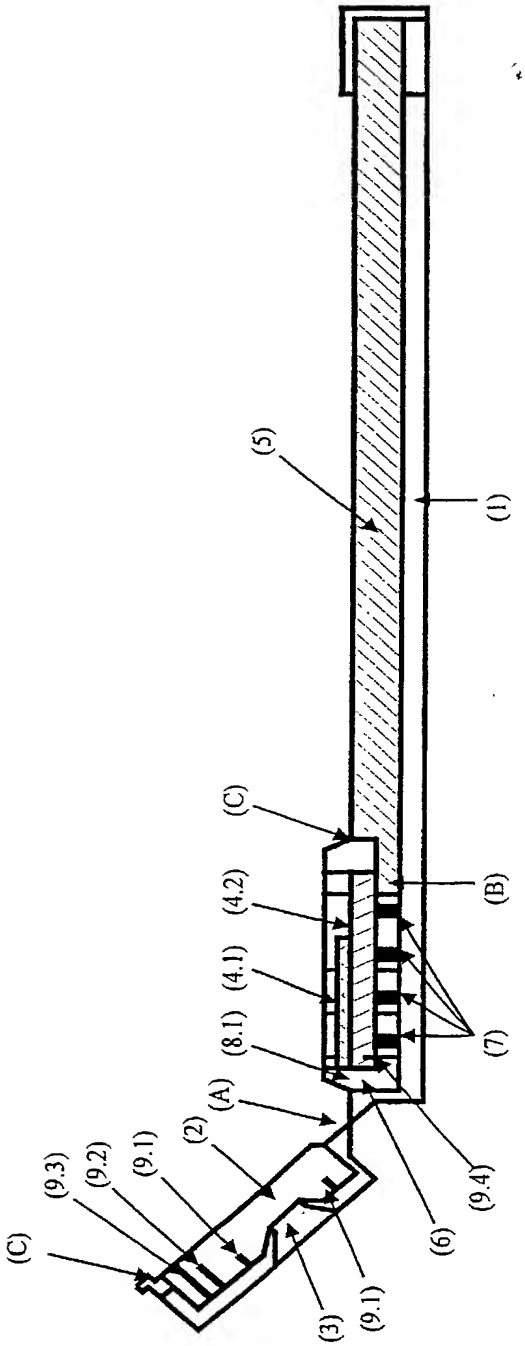


FIG. 3

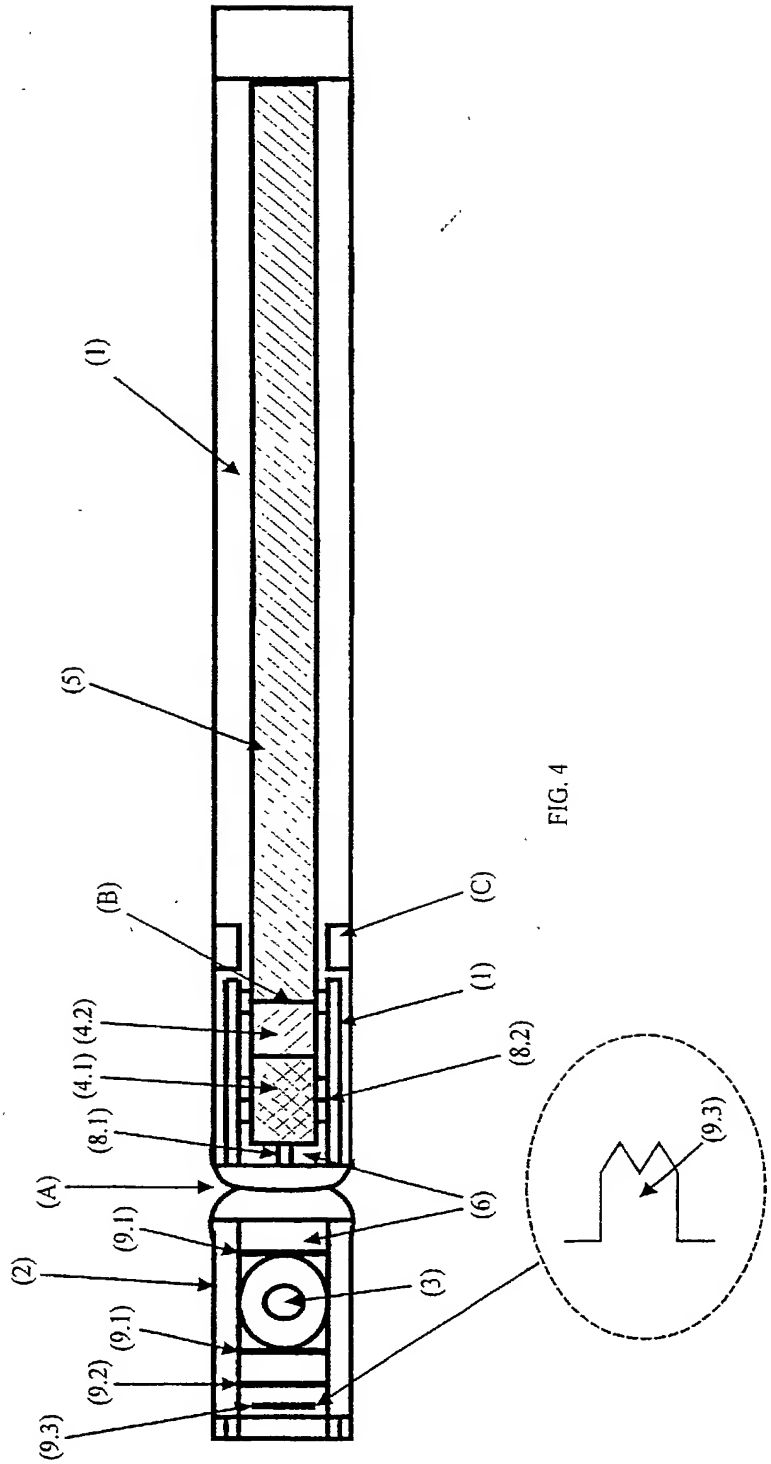


FIG. 4

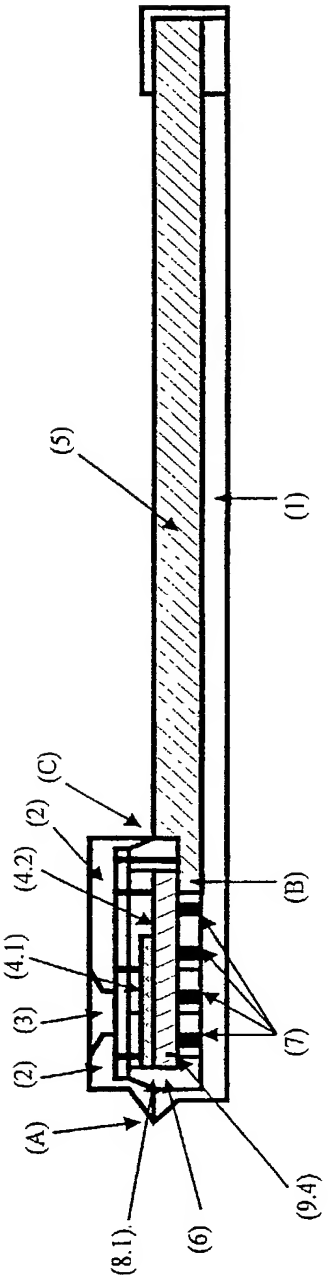


FIG. 5

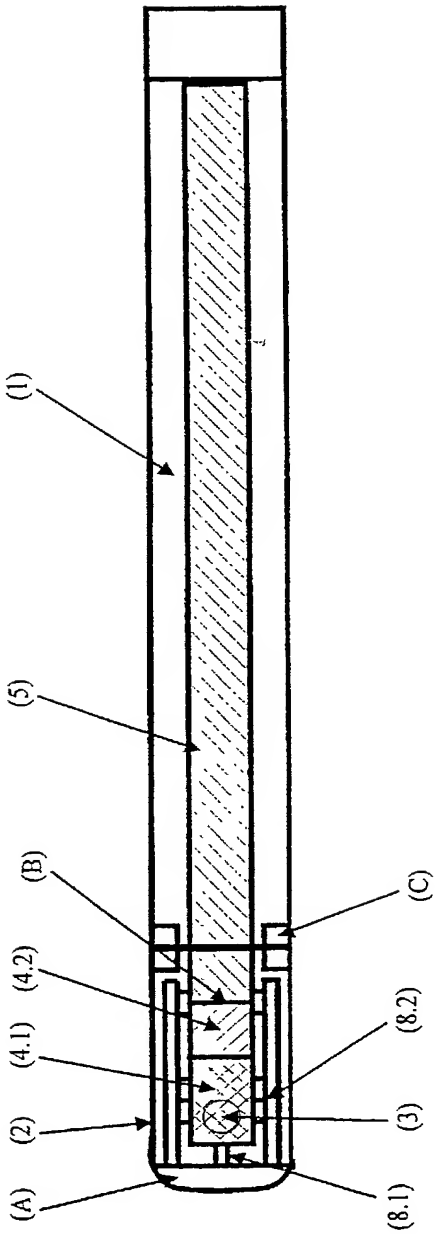


FIG. 6

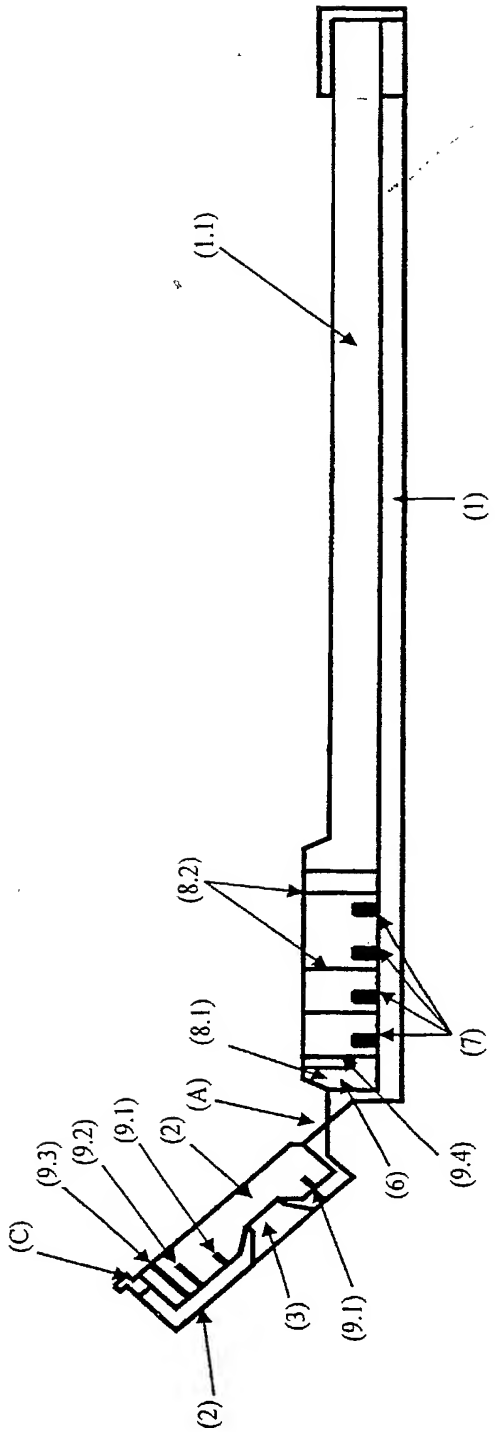


FIG. 7

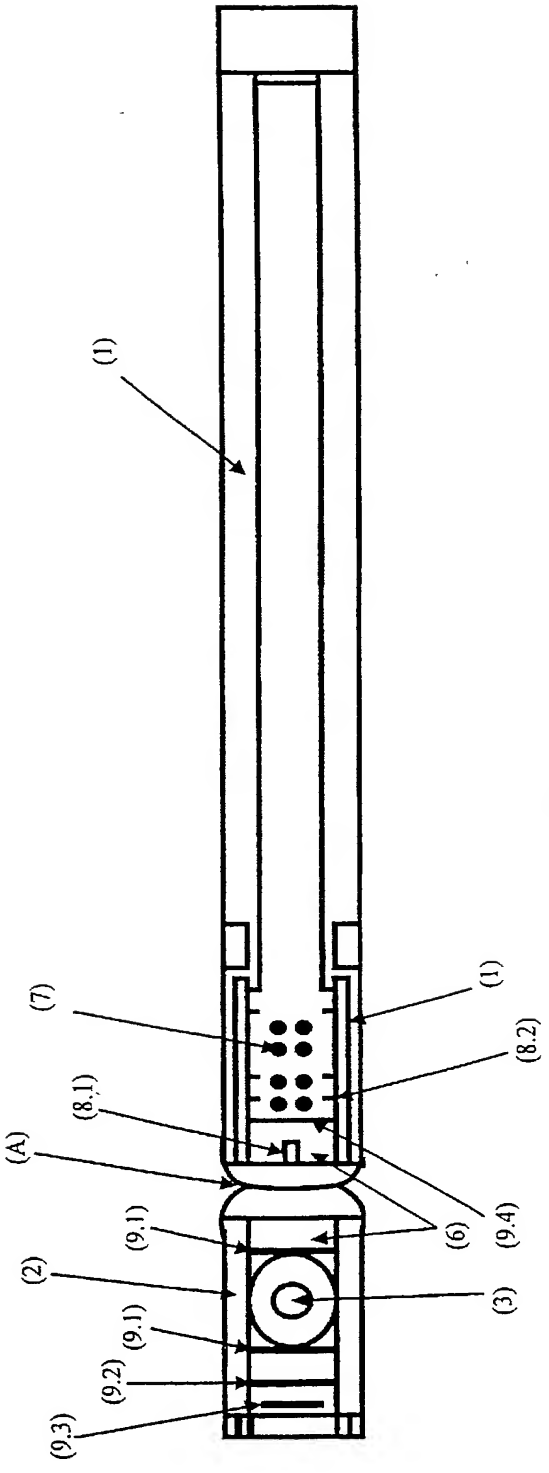


FIG. 8

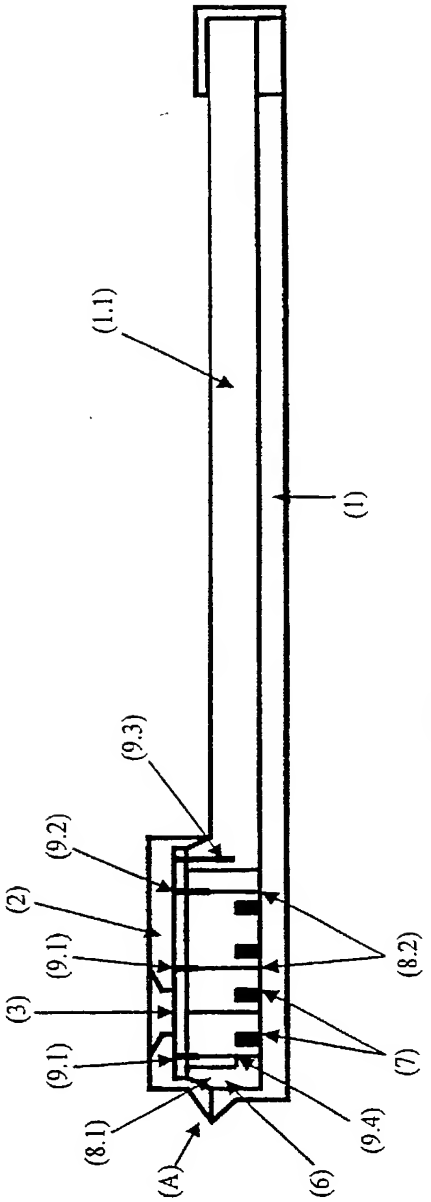


FIG. 9

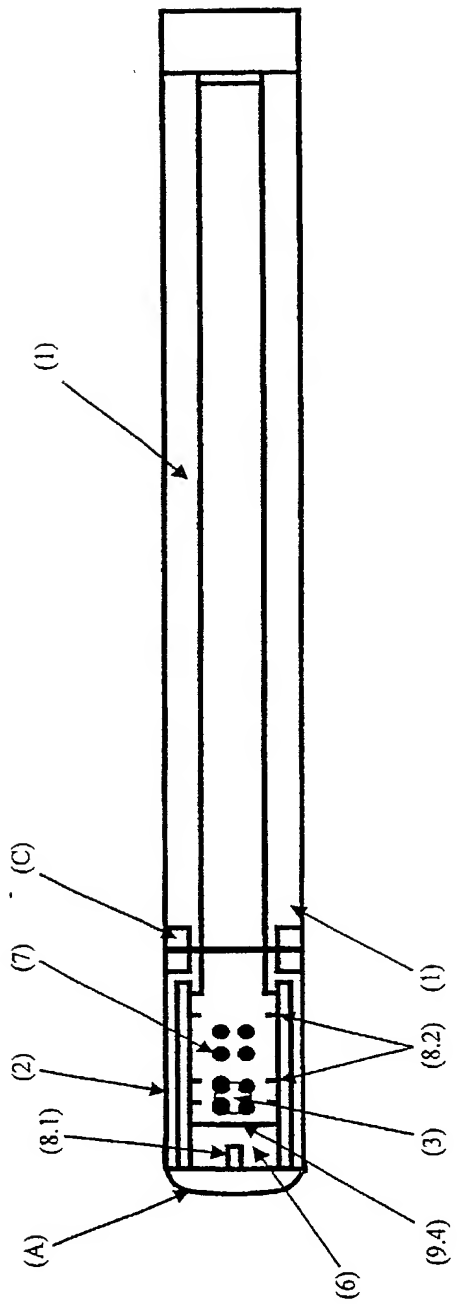


FIG. 10

3.008954 .060702

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Insert Title: A TEST STRIP PROVIDED DEVICE WITH A LID-PROVIDED PRETREATMENT PORTION

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
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United States Application Number _____;
and amended on _____ (if applicable) and/or
the specification was filed on 20 October 2000 as PCT
International Application Number PCT/FI00/00914; and was
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Prior Foreign Application(s)			Priority Claimed	
19992286 (Number)	Finland (Country)	21 October 1999 (Month/Day/Year Filed)	☑ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No

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Insert Prior U.S. Application(s): (if any)

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(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

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Full Name of First or Sole Inventor: Insert Name of Inventor. Insert Date This Document is Signed

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Full Name of Sixth Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

*DATE OF SIGNATURE